P21252.P01

60 Rec'd PCT/PTO 13 JUL 2001

Form PTO-1390	U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER P21252
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. APPLICATION NO. (If known, see 37 CFR 1.5) 09/868924
INTERNATIONAL APPLICATION NO. PCT/JP00/00245	INTERNATIONAL FILING DATE 20 January 2000	PRIORITY DATE CLAIMED 20 January 1999

TITLE OF INVENTION

PROCESS FOR PRODUCING HMG-CoA REDUCTASE INHIBITORS

APPLICANT(S) FOR DO/EO/US

Shinichi HASHIMOTO, Yoshiyuki YONETANI, and Akio OZAKI

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information.

1. X This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.
2. __ This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. X This is an express request to promptly begin national examination procedures (35 U.S.C. 371(f)).
4. X The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).
5. X A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. X is attached hereto (required only if not communicated by the International Bureau).
   b. X has been communicated by the International Bureau.
   c. __ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. X An English language translation of the International Application as filed (35 U.S.C. 371 (c)(2)).
7. __ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. __ are attached hereto (required only if not communicated by the International Bureau).
   b. __ have been communicated by the International Bureau.
   c. __ have not been made; however, the time limit for making such amendments has NOT expired.
   d. __ have not been made and will not be made.
8. __ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3))
9. X An oath or declaration of the inventor(s) (35 U.S.C. .371(c)(4)). "Unexecuted"
10. __ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (U.S.C. 371(c)(5)).

Items 11 to 16 below concern other document(s) or information included:

11. Assignee: KYOWA HAKKO KOGYO CO., LTD. Of Tokyo, JAPAN
12. __ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
13. __ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
14. __ A FIRST preliminary amendment.
    __ A SECOND or SUBSEQUENT preliminary amendment.
15. __ A substitute specification.
16. __ A change of power of attorney and/or address letter.
17. __ Figure of Drawing to be published________
18. X Other items or information:
    Cover Sheet and International Application as published (in Japanese).
    PCT/RO/101-PCT Request(in English and Japanese).
    PCT/IPEA/409.
    PCT/IB/301.
    PCT/IB/304.
    PCT/IB/308.
    PCT/IB/332.
    PCT/IB/338.
    PCT/ISA/210(in English and Japanese).
    Cover Letter under 35 USC 371 and 1.495.
    Claim of Priority.

JC18 Rec'd PCT/PTO 13 JUL 2001

U.S. APPLICATION NO. (If known, see 37 CFR 1.5) 09/868924	INTERNATIONAL APPLICATION NO. PCT/JP00/00245	ATTORNEY'S DOCKET NUMBER P21252	
19. __ The following fees are submitted:		CALCULATIONS	PTO USE ONLY

Basic National Fee (37 CFR 1.492(a)(1)-(5)):

Search report has been prepared by the EPO or JPO. . . . . $ 860.00

International preliminary examination fee paid to USPTO (37 CFR 1.482). . . . . $ 690.00

No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO(37 CFR 1.445(a)(2). $ 710.00

Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO. $1,000.00

International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4). . . . .$ 100.00

				CALCULATIONS	PTO USE ONLY
ENTER APPROPRIATE BASIC FEE AMOUNT =				$860.00	
Surcharge of $130.00 for furnishing the oath or declaration later than __ 20 __ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				$	
Claims	Number Filed		Number Extra	RATE		
Total Claims	9 - 20 =		0	X $18.00	$0.00	
Independent Claims	1 - 3 =		0	X $80.00	$0.00	
Multiple dependent claim(s) (if applicable)				+ $270.00	$0.00	
TOTAL OF ABOVE CALCULATIONS =					$860.00	
__ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by ½.					$	
SUBTOTAL =					$860.00	
Processing fee of $130.00 for furnishing the English translation later than __ 20 __ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +						
Extension of Time fee in the amount of $						
TOTAL NATIONAL FEE =					$860.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +						
TOTAL FEES ENCLOSED =					$860.00	
					Amount to be refunded	$
					Charged	$

a. _X_ A check in the amount of $860.00 to cover the above fees is enclosed.

b. ___ Please charge my Deposit Account No. _____ in the amount of $_____ to cover the above fees.

c. _X_ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 19-0089.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO **CUSTOMER NO. 7055**
AT THE PRESENT ADDRESS OF:
Bruce H. Bernstein
GREENBLUM & BERNSTEIN, P.L.C.
1941 Roland Clarke Place
Reston, VA 20191
(703) 716-1191

Leslie Wagerner Reg No 33,329
SIGNATURE
Bruce H. Bernstein
NAME

29,027
REGISTRATION NUMBER

SPECIFICATION

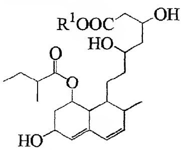
PROCESS FOR PRODUCING HMG-CoA REDUCTASE INHIBITORS

Technical Field

The present invention relates to a process for producing a compound, which inhibits hydroxymethylglutaryl CoA (HMG-CoA) reductase and has an action of reducing serum cholesterol.

Background Art

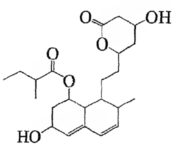
A compound represented by the formula (VI-a) (hereinafter referred to as compound (VI-a)):



(VI-a)

wherein R^1 represents a hydrogen atom or an alkali metal, or

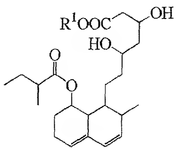
a lactone form of compound (VI-a) represented by the formula (VI-b) (hereinafter referred to as compound (VI-b)):



(VI-b)

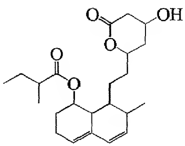
is known to inhibit HMG-CoA reductase and exhibit an action of reducing serum cholesterol and the like (*The Journal of Antibiotics*, 29, 1346 (1976)).

There have been several reports regarding a method for producing the compound (VI-a) or the compound (VI-b) from a compound represented by the formula (V-a) (hereinafter referred to as compound (V-a)):



(V-a)

wherein R^1 represents a hydrogen atom or an alkali metal, or the lactone form of compound (V-a) represented by the formula (V-b)(hereinafter referred to as compound (V-b)):



(V-b)

using a microorganism.

Specifically, Japanese Patent Application Laid-Open (kokai) No. 57-50894 describes a method which uses filamentous fungi; both Japanese Patent Application Laid-Open (kokai) No. 7-184670 and International Publication WO96/40863 describe a method which uses *Actinomycetes*; and Japanese Patent No. 2672551 describes a method which uses recombinant *Actinomycetes*. As is well known, however, since filamentous fungi and *Actinomycetes* grow with filamentous form by elongating hyphae, the viscosity of the culture in a fermentor increases. This often causes shortage of oxygen in the culture, and since the culture becomes heterogeneous, reaction efficiency tends to be reduced. In order to resolve this oxygen shortage and maintain

homogeneousness of the culture, the agitation rate of the fermentor should be raised, but by raising the agitation rate, hyphae are sheared and activity of the microorganisms tends to decrease (Basic Fermentation Engineering (Hakko Kogaku no Kiso) p.169 - 190, P.F. Stansbury, A. Whitaker, Japan Scientific Societies Press (1988)).

Furthermore, the above *Actinomycetes* and filamentous fungi have an ability to sporulate. Since spores tend to disperse much more easily than cells and have an ability of surviving even under conditions where vegetative cells perish readily, these spores tend to become a source of microorganism contamination in culturing and purification processes.

Disclosure of the Invention

The object of the present invention is to provide an industrially advantageous method for producing a compound which inhibits HMG-CoA reductase and has an action of reducing the level of serum cholesterol and the like.

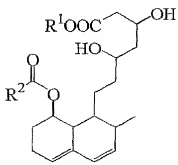
The present inventors have considered that if hydroxylation of compound (V-a) or compound (V-b) could be carried out with a microorganism having hydroxylation activity, having no ability to sporulate and showing no hyphal growth, inconvenience such as the decrease of reaction efficiency due to microorganism contamination caused by the release of spores during the production process or the heterogeneity of the culture caused by formation of hyphae could be avoided, and that this would be industrially advantageous. As a result of intensive studies directed to this object, the present inventors have accomplished the present invention.

Thus, the present invention relates to the following (1) to (9).

Hereinafter, in the formulas, R^1 represents a hydrogen atom, a substituted or unsubstituted alkyl, or an alkali metal, and R^2 represents a substituted or unsubstituted

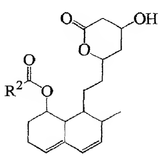
alkyl, or a substituted or unsubstituted aryl, unless otherwise specified.

(1) A process for producing a compound (II-a) or a compound (II-b) wherein a microorganism having an activity of producing compound (II-a) or a compound (II-b) from a compound (I-a) or a compound (I-b), having no ability to sporulate and showing no hyphal growth, a culture of said microorganism, or a treated product of said culture is used as an enzyme source, and the process comprises: allowing the compound (I-a) or the compound (I-b) to exist in an aqueous medium; allowing the compound (II-a) or the compound (II-b) to be produced and accumulated in said aqueous medium; and collecting the compound (II-a) or the compound (II-b) from said aqueous medium, and wherein the compound (I-a) is a compound represented by the formula (I-a) (herein referred to as compound (I-a)) :



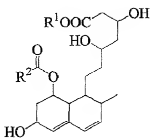
(I-a)

the compound (I-b) is a lactone form of compound (I-a) represented by the formula (I-b) (herein referred to as compound (I-b)):



(I-b)

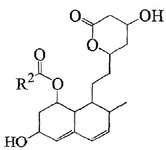
the compound (II-a) is a compound represented by the formula (II-a) (herein referred to as compound (II-a)):



(II-a)

and

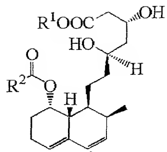
the compound (II-b) is a lactone form of compound (II-a) represented by the formula (II-b) (herein referred to as compound (II-b)):



(II-b)

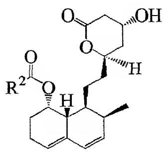
(2) The process according to (1) above, wherein

the compound (I-a) is a compound represented by the formula (III-a) (herein referred to as compound (III-a)):



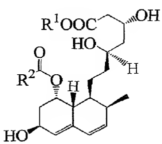
(III-a)

the compound (I-b) is a compound represented by the formula (III-b) (herein referred to as compound (III-b)):



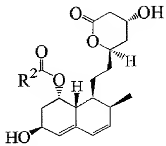
(III-b)

the compound (II-a) is a compound represented by the formula (IV-a) (herein referred to as compound (IV-a)):



(IV-a)

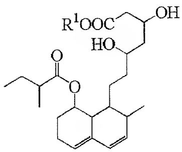
the compound (II-b) is a compound represented by the formula (IV-b) (herein referred to as compound (IV-b)):



(IV-b)

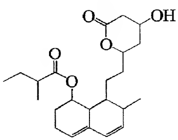
(3) The process according to (1) above, wherein

the compound (I-a) is a compound represented by the formula (V-a) (herein referred to as compound (V-a)):



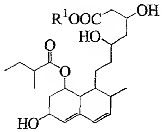
(V-a)

the compound (I-b) is a compound represented by the formula (V-b) (herein referred to as compound (V-b)):



(V-b)

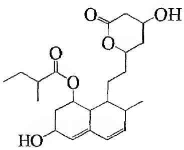
the compound (II-a) is a compound represented by the formula (VI-a) (herein referred to as compound (VI-a)):



(VI-a)

and;

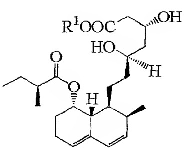
the compound (II-b) is a compound represented by the formula (VI-b) (herein referred to as compound (VI-b)):



(VI-b)

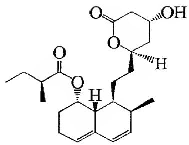
(4) The process according to (1) above, wherein

the compound (I-a) is a compound represented by the formula (VII-a) (herein referred to as compound (VII-a)):



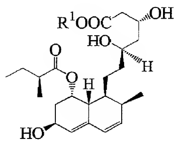
(VII-a)

the compound (I-b) is a compound represented by the formula (VII-b) (herein referred to as compound (VII-b));:



(VII-b)

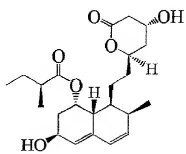
the compound (II-a) is a compound represented by the formula (VIII-a) (herein referred to as compound (VIII-a)):



(VIII-a)

and,

the compound (II-b) is a compound represented by the formula (VIII-b) (herein referred to as compound (VIII-b)):



(VIII-b)

(5) The process according to (1), wherein the treated product of the culture of the microorganism is a treated product selected from cultured cells; treated products such as dried cells, freeze-dried cells, cells treated with a surfactant, cells treated with an enzyme, cells treated by ultrasonication, cells treated by mechanical milling, cells

treated by solvent; a protein fraction of a cell; and an immobilized products of cells or treated cells.

(6) The process according to (1) above, wherein the microorganism is selected from those belonging to the genus *Mycobacterium, Corynebacterium, Brevibacterium, Rhodococcus, Gordona, Arthrobacter, Micrococcus, Cellulomonas* and *Sphingomonas*.

(7) The process according to (1) above, wherein the microorganism is one selected from *Mycobacterium phlei, Mycobacterium smegmatis, Mycobacterium thermoresistibile, Mycobacterium neoaurum, Mycobacterium parafortuitum, Mycobacterium gilvum, Rhodococcus globerulus, Rhodococcus equi, Rhodococcus erythropolis, Rhodococcus rhodochrous, Rhodococcus rhodnii, Rhodococcus ruber, Rhodococcus coprophilus, Rhodococcus fascians, Gordona amarae, Gordona rubropertinctus, Gordona bronchialis, Gordona sputi, Gordona aichiensis, Gordona terrae, Corynebacterium glutamicum, Corynebacterium mycetoides, Corynebacterium variabilis, Corynebacterium ammoniagenes, Arthrobacter crystallopoietes, Arthrobacter duodecadis, Arthrobacter ramosus, Arthrobacter sulfureus, Arthrobacter aurescens, Arthrobacter citreus, Arthrobacter globiformis, Brevibacterium acetylicum, Brevibacterium linens, Brevibacterium incertum, Brevibacterium iodinum, Micrococcus luteus, Micrococcus roseus, Cellulomonas cellulans, Cellulomonas cartae, Sphingomonas paucimobilis, Sphingomonas adhaesiva*, and *Sphingomonas terrae*.

(8) The process according to (1) above, wherein the microorganism is one selected from *Mycobacterium phlei* JCM5865, *Mycobacterium smegmatis* JCM5866, *Mycobacterium thermoresistibile* JCM6362, *Mycobacterium neoaurum* JCM6365, *Mycobacterium parafortuitum* JCM6367, *Mycobacterium gilvum* JCM6395, *Rhodococcus globerulus* ATCC25714, *Rhodococcus equi* ATCC21387, *Rhodococcus equi* ATCC7005, *Rhodococcus erythropolis* ATCC4277, *Rhodococcus rhodochrous* ATCC21430, *Rhodococcus rhodochrous* ATCC13808, *Rhodococcus rhodnii*

ATCC35071, *Rhodococcus ruber* JCM3205, *Rhodococcus coprophilus* ATCC29080, *Rhodococcus fascians* ATCC12974, *Rhodococcus fascians* ATCC35014, *Gordona amarae* ATCC27808, *Gordona rubropertinctus* IFM-33, *Gordona rubropertinctus* ATCC14352, *Gordona bronchialis* ATCC25592, *Gordona sputi* ATCC29627, *Gordona aichiensis* ATCC33611, *Gordona terrae* ATCC25594, *Corynebacterium glutamicum* ATCC13032, *Corynebacterium glutamicum* ATCC14020, *Corynebacterium glutamicum* ATCC19240, *Corynebacterium mycetoides* ATCC21134, *Corynebacterium variabilis* ATCC15753, *Corynebacterium ammoniagenes* ATCC6872, *Arthrobacter crystallopoietes* ATCC15481, *Arthrobacter duodecadis* ATCC13347, *Arthrobacter ramosus* ATCC13727, *Arthrobacter sulfureus* ATCC19098, *Arthrobacter aurescens* ATCC13344, *Arthrobacter citreus* ATCC11624, *Arthrobacter globiformis* ATCC8010, *Brevibacterium acetylicum* ATCC953, *Brevibacterium linens* ATCC19391, *Brevibacterium linens* ATCC9172, *Brevibacterium incertum* ATCC8363, *Brevibacterium iodinum* IFO3558, *Micrococcus luteus* ATCC4698, *Micrococcus roseus* ATCC186, *Cellulomonas cellulans* ATCC15921, *Cellulomonas cartae* ATCC21681, *Sphingomonas paucimobilis* ATCC29837, *Sphingomonas adhaesiva* JCM7370, and *Sphingomonas terrae* ATCC15098.

(9) The process according to (1) above, wherein the microorganism is *Gordona* sp. ATCC19067.

The present invention is described in detail below.

Examples of an enzyme source used in the present invention include: a microorganism which has an activity of producing the above compound (II-a) or the above compound (II-b) from the above compounds (I-a) or the above compound (I-b), having no ability to sporulate and showing no hyphal growth; a culture of said microorganism; or a treated product of said culture.

Alkyl is a linear or branched alkyl containing 1 to 10 carbon atoms, preferably 1 to 6 carbon atoms, such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, neopentyl, hexyl, isohexyl, heptyl, 4,4-dimethylpentyl, octyl, 2,2,4-trimethylpentyl, nonyl, decyl, and various branched chain isomers thereof.

Examples of aryl include phenyl and naphtyl.

The substituent of the substituted alkyl may be 1 to 3 identical or different groups, and examples thereof include halogens, hydroxy, amino, alkoxy and aryl.

The substituent of the substituted aryl may be 1 to 3 identical or different groups, and examples thereof include halogens, hydroxy, amino, alkyl and alkoxy.

The alkyl moiety of the alkoxy has the same definition as in the alkyl mentioned above.

Alkali metal represents each element of lithium, sodium, potassium, rubidium, cesium or francium.

Examples of the above microorganism include microorganisms selected from the genus *Mycobacterium, Corynebacterium, Brevibacterium, Rhodococcus, Gordona, Arthrobacter, Micrococcus, Cellulomonas* and *Sphingomonas.*

Specific examples include microorganisms selected from *Mycobacterium phlei, Mycobacterium smegmatis, Mycobacterium thermoresistibile, Mycobacterium neoaurum, Mycobacterium parafortuitum, Mycobacterium gilvum, Rhodococcus globerulus, Rhodococcus equi, Rhodococcus erythropolis, Rhodococcus rhodochrous, Rhodococcus rhodnii, Rhodococcus ruber, Rhodococcus coprophilus, Rhodococcus fascians, Gordona amarae, Gordona rubropertinctus, Gordona bronchialis, Gordona*

sputi, Gordona aichiensis, Gordona terrae, Corynebacterium glutamicum, Corynebacterium mycetoides, Corynebacterium variabilis, Corynebacterium ammoniagenes, Arthrobacter crystallopoietes, Arthrobacter duodecadis, Arthrobacter ramosus, Arthrobacter sulfureus, Arthrobacter aurescens, Arthrobacter citreus, Arthrobacter globiformis, Brevibacterium acetylicum, Brevibacterium linens, Brevibacterium incertum, Brevibacterium iodinum, Micrococcus luteus, Micrococcus roseus, Cellulomonas cellulans, Cellulomonas cartae, Sphingomonas paucimobilis, Sphingomonas adhaesiva, and *Sphingomonas terrae.*

More specifically, examples include *Mycobacterium phlei* JCM5865, *Mycobacterium smegmatis* JCM5866, *Mycobacterium thermoresistibile* JCM6362, *Mycobacterium neoaurum* JCM6365, *Mycobacterium parafortuitum* JCM6367, *Mycobacterium gilvum* JCM6395, *Rhodococcus globerulus* ATCC25714, *Rhodococcus equi* ATCC21387, *Rhodococcus equi* ATCC7005, *Rhodococcus erythropolis* ATCC4277, *Rhodococcus rhodochrous* ATCC21430, *Rhodococcus rhodochrous* ATCC13808, *Rhodococcus rhodnii* ATCC35071, *Rhodococcus ruber* JCM3205, *Rhodococcus coprophilus* ATCC29080, *Rhodococcus fascians* ATCC12974, *Rhodococcus fascians* ATCC35014, *Gordona amarae* ATCC27808, *Gordona rubropertinctus* IFM-33, *Gordona rubropertinctus* ATCC14352, *Gordona bronchialis* ATCC25592, *Gordona sputi* ATCC29627, *Gordona aichiensis* ATCC33611, *Gordona terrae* ATCC25594, *Corynebacterium glutamicum* ATCC13032, *Corynebacterium glutamicum* ATCC14020, *Corynebacterium glutamicum* ATCC19240, *Corynebacterium mycetoides* ATCC21134, *Corynebacterium variabilis* ATCC15753, *Corynebacterium ammoniagenes* ATCC6872, *Arthrobacter crystallopoietes* ATCC15481, *Arthrobacter duodecadis* ATCC13347, *Arthrobacter ramosus* ATCC13727, *Arthrobacter sulfureus* ATCC19098, *Arthrobacter aurescens* ATCC13344, *Arthrobacter citreus* ATCC11624, *Arthrobacter globiformis* ATCC8010, *Brevibacterium acetylicum* ATCC953, *Brevibacterium linens* ATCC19391, *Brevibacterium linens* ATCC9172, *Brevibacterium incertum* ATCC8363, *Brevibacterium iodinum* IFO3558, *Micrococcus luteus* ATCC4698, *Micrococcus roseus*

ATCC186, *Cellulomonas cellulans* ATCC15921, *Cellulomonas cartae* ATCC21681, *Sphingomonas paucimobilis* ATCC29837, *Sphingomonas adhaesiva* JCM7370, *Sphingomonas terrae* ATCC15098 and *Gordona* sp. ATCC19067.

In addition, a subculture, mutant, derivative or recombinant produced by a recombinant DNA technique of any of these microorganisms can also be used.

As a medium used for the culture of the microorganism used in the present invention, both natural and synthetic media can be used, as long as the media contain a carbon source, a nitrogen source, inorganic salts and the like which can be assimilated by the microorganism of the present invention, and can achieve an efficient culture of the microorganism of the present invention.

Specific examples of the carbon source in a medium include carbohydrates such as glucose, fructose, glycerol, maltose, starch and saccharose, and organic acids such as acetic acid and citric acid and molasses.

Specific examples of the nitrogen source include ammonia; ammonium salts of various types of inorganic acids and organic acids, such as ammonium chloride, ammonium sulfate, ammonium acetate, ammonium nitrate and ammonium phosphate; peptone, meat extract, corn steep liquor, casein hydrolysate, soybean meal, cottonseed meal, fish meal, various types of fermented microbial cells and digests thereof.

Specific examples of inorganic substances include potassium dihydrogenphosphate, dipotassium hydrogenphosphate, magnesium phosphate, magnesium sulfate, sodium chloride, ferrous sulfate, manganese sulfate, copper sulfate, and calcium carbonate.

Vitamins such as thiamin and biotin, amino acids such as glutamic acid and

aspartic acid, nucleic acid-related substances such as adenine and guanine may be added, as required.

The culturing of the microorganism used in the present invention is preferably carried out under aerobic conditions such as a shaking culture, an aeration-agitation culture or the like. Where the aeration-agitation culture is applied, it is preferred to add an appropriate amount of antifoaming agent to prevent foaming. The culture is carried out usually at 20 to 50°C, preferably at 25 to 40°C, for 6 to 120 hours. During culturing, pH is maintained at 5.0 to 10.0, preferably at 6.0 to 8.5. The pH control is carried out by using inorganic or organic acid, an alkaline solution, urea, calcium carbonate, ammonia, etc.

Examples of a treated product of the thus-obtained cultured microorganism include cultured cells; a treated product such as dried cells, freeze-dried cells, cells treated with a surfactant, cells treated with an enzyme, cells treated by ultrasonication, cells treated by mechanical milling, cell treated by solvent; a protein fraction of cells; and an immobilized product of cells or treated cells.

The methods for converting compound (I-a) or compound (I-b) into compound (II-a) or compound (II-b) may be a method of previously adding compound (I-a) or compound (I-b) to a medium in which a microorganism is to be cultured, or may be a method of adding compound (I-a) or compound (I-b) during culturing. Further, a method of allowing an enzyme source to act upon compound (I-a) or compound (I-b) in an aqueous medium may also be used.

In a case where compound (I-a) or compound (I-b) is added to a medium in which a microorganism is to be cultured, 0.1 to 10mg, preferably 0.2 to 1mg of the compound (I-a) or the compound (I-b) is added to 1 ml of medium at the beginning of or at some midpoint of the culture. When compound (I-a) or compound (I-b) is added,

it may be added after it is dissolved in a solvent such as methyl alcohol or ethyl alcohol.

In a case where a method of allowing an enzyme source to act upon compound (I-a) or compound (I-b) in an aqueous medium, the amount of enzyme to be used depends on the specific activity of the enzyme source or the like. For example, when a culture of a microorganism or a treated product of the culture is used as an enzyme source, 5 to 1,000mg, preferably 10 to 400mg of enzyme source is added per 1mg of compound (I-a) or compound (I-b). The reaction is performed in an aqueous medium, preferably at 20 to 50°C, and particularly preferably at 25 to 40°C. The reaction period depends on the amount, specific activity, etc. of the enzyme source to be used, but it is usually 0.5 to 150 hours, preferably 1 to 72 hours.

Examples of an aqueous medium include water or buffers such as phosphate buffer, HEPES (N-2 hydroxyethylpiperazine-N-ethanesulfonate) buffer and Tris (tris(hydroxymethyl)aminomethane)hydrochloride buffer. An organic solvent may be added to the above buffers, unless it inhibits reaction. Examples of organic solvent include acetone, ethyl acetate, dimethyl sulfoxide, xylene, methyl alcohol, ethyl alcohol and butanol. A mixture of an organic solvent and an aqueous medium is preferably used when compound (I-b) is used.

According to the above production method, compound (II-a) or a mixture of compound (II-a) and compound (II-b) can be obtained from compound (I-a).

Similarly, compound (II-b) or a mixture of compound (II-a) and compound (II-b) can be obtained from compound (I-b).

Moreover, a mixture of compound (II-a) and compound (II-b) can be obtained from a mixture of compound (I-a) and compound (I-b).

Compound (I-b) and compound (II-b) can easily be converted into compound (I-a) and compound (II-a) respectively, by a method for opening a lactone ring as mentioned below. Likewise, compound (I-a) and compound (II-a) can easily be converted into compound (I-b) and compound (II-b) respectively, by a method for producing lactone as mentioned below.

Examples of a method for opening a lactone ring include a method which comprises dissolving compound (I-b) or compound (II-b) in an aqueous medium and adding thereto an acid or alkali. Examples of the aqueous medium include water and an aqueous solution containing salts, which does not inhibit the reaction, such as phosphate buffer, Tris buffer and the like. The above aqueous solution may contain an organic solvent such as methanol, ethanol, ethyl acetate and the like in a concentration which does not inhibit the reaction. Examples of acid include acetic acid, hydrochloric acid and sulfuric acid, and examples of alkali include sodium hydroxide, potassium hydroxide and ammonia.

Examples of a method for producing lactone include a method which comprises dissolving compound (I-a) or compound (II-a) in a non-aqueous solvent and adding thereto an acid or base catalyst. As long as the non-aqueous solvent is an organic solvent which does not substantially contain water and is capable of dissolving compound (I-a) or compound (II-a), any type of non-aqueous solvent can be used.

Examples of non-aqueous solvents include dichloromethane and ethyl acetate. As a catalyst, any catalyst can be used, as long as it catalyzes lactonization and does not show any actions other than lactonization on a substrate or a reaction product. Examples of the above catalyst include trifluoroacetic acid and p-toluenesulfonic acid. Reaction temperature is not particularly limited, but is preferably 0 to 100°C, and is more preferably 20 to 80°C.

After completion of the reaction, compound (II-a) or compound (II-b) can be collected from the above solution by ordinary methods used in the field of organic synthetic chemistry such as extraction with organic solvents, crystallization, thin-layer chromatography, high performance liquid chromatography, etc.

As a method for detecting and quantifying the compound (II-a) or the compound (II-b) obtained by the present invention, any method can be used, as long as the detection or quantification of compound (II-a) and/or compound (II-b) can be performed. Examples thereof include ^{13}C-NMR spectroscopy, ^{1}H-NMR spectroscopy, mass spectroscopy, high performance liquid chromatography (HPLC) etc.

There may be stereoisomers such as optical isomers for some compounds among compound (I-a), compound (I-b), compound (II-a) and compound (II-b). The present invention covers all possible isomers and mixtures thereof including these stereoisomers.

As compound (I-a), compound (III-a) is preferable, compound (V-a) is more preferable, and compound (VII-a) is particularly preferable.

As compound (I-b), compound (III-b) is preferable, compound (V-b) is more preferable, and compound (VII-b) is particularly preferable.

As compound (II-a), compound (IV-a) is preferable, compound (VI-a) is more preferable, and compound (VIII-a) is particularly preferable.

As compound (II-b), compound (IV-b) is preferable, compound (VI-b) is more preferable, and compound (VIII-b) is particularly preferable.

The examples of the present invention is described below, but the present

invention is not limited to these examples.

The Best Mode for Carrying out the Invention

Example 1.

100mg of compound (VII-b) (produced by Sigma) was dissolved in 9.5ml of methanol, and 0.5ml of 1mol/l sodium hydroxide was added. The mixture was stirred at room temperature for 1 hour. The obtained reaction solution was dried to be solidified, and was dissolved by adding 5ml of deionized water, followed by adjusting pH to around 6.5 to 7.5 with about 0.1ml of 1mol/l hydrochloric acid. Then, 4.9ml of deionized water was added to the mixture to obtain 10ml of compound (VII-a), whose final concentration was 10mg/ml (a compound wherein, in formula (VII-a), R^1 is sodium).

Various types of microorganisms shown in Tables 1 and 2 were independently plated onto an agar medium (1% peptone (produced by Kyokuto Pharmaceutical Industrial Co., Ltd.), 0.7% meat extract (produced by Kyokuto Pharmaceutical Industrial Co., Ltd.), 0.3% NaCl, 0.2% yeast extract (produced by Nihon Pharmaceutical Co., Ltd.), 2% bacto agar (produced by Difco), adjusted to pH7.2 with 1mol/l sodium hydroxide), then cultured for 3 days at each temperature shown in Tables 1 and 2. An inoculating loop of each of the strains which grew on the agar medium was inoculated into a test tube containing 3ml of LB medium (1% bacto tryptone (produced by Difco), 0.5% bacto yeast extract (produced by Difco), adjusted to pH7.2 with 1mol/l sodium hydroxide). This tube was then subjected to shaking culture for 24 hours at each temperature shown in Tables 1 and 2. After culturing, 0.25ml of the culture was inoculated in test tubes containing 5ml of TB medium (1.4% bacto tryptone (produced by Difco), 2.4% bacto yeast extract (produced by Difco), 0.231% KH_2PO_4, 1.251% K_2HPO_4, adjusted to pH7.4 with 1mol/l sodium hydroxide). The tubes were then subjected to shaking culture for 24 hours at each temperature shown in Tables 1 and 2. After 24 hours, the above-obtained compound (VII-a) was added to

each of test tubes in a the final concentration of 0.4mg/ml, and then reaction was performed with shaking at each temperature shown in Tables 1 and 2 for 48 hours.

After completion of the reaction, the reaction solution was adjusted to pH3.5 with acetic acid. 1 ml of ethyl acetate was added to 0.5ml of this reaction solution followed by shaking for 1 hour. After shaking, the reaction solution was separated into 2 layers by centrifugation at 3,000rpm for 5 minutes, then the upper ethyl acetate layer was collected. The solvent was removed with a centrifugal evaporator, and the residue was dissolved in 0.5ml of methanol. Using a portion of this methanol solution, HPLC analysis was carried out (Column: Inertsil ODS-2 (5μm, 4×250mm, produced by GL Science), Column temperature: 60°C, Mobile phase: acetonitrile:water:phosphoric acid = 55:45:0.05, Flow rate: 0.9ml/min, Detection wavelength: 237nm), to detect and quantify compound (VIII-a) (a compound wherein, in formula (VIII-a), R^1 is sodium). The results are shown in Tables 1 and 2.

Table 1

Strain		Compound (VIII-a) mg/l	Culturing Temperature (℃)
Mycobacterium phlei	JCM 5865	1.6	37
Mycobacterium smegmatis	JCM 5866	0.4	37
Mycobacterium thermoresistibile	JCM 6362	9.1	37
Mycobacterium neoaurum	JCM 6365	3.7	37
Mycobacterium parafortuitum	JCM 6367	7.4	37
Mycobacterium gilvum	JCM 6395	9.6	37
Rhodococcus globerulus	ATCC25714	4.9	28
Rhodococcus equi	ATCC21387	2.5	30
Rhodococcus erythropolis	ATCC4277	1.4	30
Rhodococcus rhodochrous	ATCC21430	4.9	30
Rhodococcus equi	ATCC7005	1.4	30
Rhodococcus rhodochrous	ATCC13808	4.7	28
Rhodococcus rhodnii	ATCC35071	0.4	28
Rhodococcus ruber	JCM 3205	0.6	28
Rhodocuccus coprophilus	ATCC29080	5.6	28
Rhodococcus fascians	ATCC12974	1.3	28
Rhodococcus fascians	ATCC35014	5.2	30
Gordona amarae	ATCC27808	1.2	30
Gordona rubropertinctus	IFM-33	2.5	30
Gordona bronchialis	ATCC25592	0.9	28
Gordona rubropertinctus	ATCC14352	0.7	28
Gordona sputi	ATCC29627	0.3	28
Gordona aichiensis	ATCC33611	0.6	28
Gordona sp.	ATCC19067	4.0	30
Gordona terrae	ATCC25594	0.3	28

Table 2

Strain		Compound (VIII-a) mg/l	Culturing Temperature (°C)
Corynebacterium glutamicum	ATCC13032	1.1	30
Corynebacterium glutamicum	ATCC14020	0.7	30
Corynebacterium glutamicum	ATCC19240	1.0	30
Corynebacterium mycetoides	ATCC21134	0.3	30
Corynebacterium variabilis	ATCC15753	1.7	30
Corynebacterium ammoniagenes	ATCC6872	0.6	30
Arthrobacter crystallopoietes	ATCC15481	0.5	30
Arthrobacter duodecadis	ATCC13347	0.7	30
Arthrobacter ramosus	ATCC13727	2.2	30
Arthrobacter sulfureus	ATCC19098	1.1	30
Arthrobacter aurescens	ATCC13344	1.3	30
Arthrobacter citreus	ATCC11624	1.2	30
Arthrobacter globiformis	ATCC8010	0.3	30
Brevibacterium acetylicum	ATCC953	0.4	30
Brevibacterium linens	ATCC19391	0.5	30
Brevibacterium linens	ATCC9172	0.6	30
Brevibacterium incertum	ATCC8363	0.5	30
Brevibacterium iodinum	IF03558	0.8	30
Micrococcus luteus	ATCC4698	0.5	30
Micrococcus roseus	ATCC186	0.4	30
Cellulomonas cellulans	ATCC15921	0.7	30
Cellulomonas cartae	ATCC21681	0.7	30
Sphingomonas paucimobilis	ATCC29837	3.4	30
Sphingomonas adhaesiva	JCM 7370	2.7	37
Sphingomonas terrae	ATCC15098	3.1	30

Example 2.

Mycobacterium gilvum JCM 6395 strain was plated onto the same agar medium as in Example 1 and was cultured at 37°C for 3 days. The strain which grew on the agar medium was inoculated into 4 test tubes each containing 3ml of LB medium, followed by shaking culture at 37°C for 24 hours. 1.25ml of each of the cultures was inoculated into eight 300-ml Erlenmeyer flasks containing 25ml of TB medium,

followed by shaking culture at 37°C. After 24 hours, compound (VII-a) prepared as in Example 1 (a compound wherein, in formula (VII-a), R^1 is sodium) was added in the final concentration of 0.4mg/ml, and the mixture was shaken at 37°C for 48 hours. After completion of the reaction, the culture was centrifuged at 3,000rpm at 4°C for 10 minutes to collect the supernatant. The pH of this supernatant was adjusted to 3.5 with acetic acid. After 400ml of ethyl acetate was added thereto, the mixture was shaken at 30°C for 1 hour. After leaving to stand, supernatant was collected. The same operation was repeated to the aqueous lower layer, then the obtained ethyl acetate layer was combined with the aforementioned supernatant. After 100ml of saturated saline solution was added to this ethyl acetate layer, the mixture was shaken, and supernatant was collected.

Next, 5g of anhydrous Na_2SO_4 was added to this supernatant and the mixture was left at room temperature for 15 minutes. Then, ethyl acetate was evaporated under reduced pressure so that the mixture was solidified. The obtained residue was dissolved in 5ml of deionized water, and pH was adjusted to 9.0 with sodium hydroxide, followed by passing the solution through a 50ml HP-20 column (25×100mm, produced by Mitsubishi Chemical Corp.) After washing the column with 150ml of deionized water, elution was carried out in a stepwise manner with 100ml of acetone solutions each of which contains 20%, 30% and 40% acetone. The collected fractions were subjected to the same HPLC analysis as in Example 1, thereby recovering a fraction containing compound (VIII-a). Acetonitrile was removed from this fraction under reduced pressure, then pH of the solution was adjusted to 3.0 with 1mol/l hydrochloric acid. After 360ml of ethyl acetate was added to this solution, the mixture was shaken. After leaving to stand, supernatant was collected. After 90ml of saturated saline solution was added to this supernatant, the mixture was shaken, and left to stand, and the supernatant was collected.

Subsequently, 4.5g of anhydrous Na_2SO_4 was added to this supernatant and

the mixture was left at room temperature for 15 minutes followed by evaporating to dryness under reduced pressure. The obtained dried residue was dissolved in dichloromethane and lactonized by adding 1% trifluoroacetic acid. This reaction product was fractionated with preparative TLC (Silica gel plate: No.1.05744 (200×200mm, thickness: 0.5mm, produced by Merck), development solvent: ethyl acetate, color-development solution: 12.5% phosphomolybdic acid-1% cerium/10% sulfuric acid solution), thereby obtaining 0.8mg of compound (VIII-b). The results of mass spectrum and ^{1}H-NMR spectrum analyses of the obtained compound (VIII-b) are as follows.

Mass Spectrum

Applying JMS-HX/HX110A mass spectrometer (manufactured by NIHON DENSHI Ltd.), the measurement was done in a positive mode using m-nitrobenzyl alcohol as a matrix. As a result, a pseudoion peak ($[M+H]^+$) was obtained at m/z 407, and the actual measurement value matched with the value expected from the structure and molecular weight (406) of compound (II-b).

^{1}H-NMR spectrum

Applying type JNM-α400 spectrometer (manufactured by NIHON DENSHI Ltd.), the measurement was done at 400MHz in duetero chloroform, using TMS as an internal standard. The results are shown below. The spectrum data were consistent with the known data regarding compound (VIII-b) (*Sankyo Research Laboratories Annual Report*, 37, 147 (1985).

δ ppm($CDCl_3$):6.01(1H, d,J=9.5Hz), 5.89(1H, dd,J=9.5, 5.9Hz), 5.58(1H, m), 5.41(1H, m), 4.60(1H, ddd,J=10.6, 7.3, 5.4, 2.8Hz), 4.40(1H, m), 4.38(1H, m), 2.74(1H, dd,J=13.1, 6.0, 4.8, 1.5Hz), 2.40(1H, m), 2.36(1H, m), 2.34(1H, m), 1.95(1H, dddd, J=14.4, 3.7, 2.9, 1.7Hz), 1.86(1H, dddd, J=12.5, 12.3, 7.3, 4.3Hz), 1.69(1H, m), 1.68(1H, m), 1.64(1H, m), 1.57(1H, m), 1.5-1.4(2H, m), 1.43(1h, m), 1.30(1H, m), 1.12(3H, d,

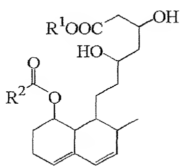
J=6.8Hz), 0.91(3H, d, J=7.1Hz), 0.89(3H, t, J=7.4Hz)

Industrial Applicability

According to the present invention, it becomes possible to efficiently produce a compound, which inhibits HMG-CoA reductase and has an action of reducing the level of serum cholesterol.

CLAIMS

1. A process for producing a compound (II-a) or a compound (II-b) wherein a microorganism having an activity of producing compound (II-a) or a compound (II-b) from a compound (I-a) or a compound (I-b), having no ability to sporulate and showing no hyphal growth, a culture of said microorganism, or a treated product of said culture is used as an enzyme source, and the process comprises: allowing the compound (I-a) or the compound (I-b) to exist in an aqueous medium; allowing the compound (II-a) or the compound (II-b) to be produced and accumulated in said aqueous medium; and collecting the compound (II-a) or the compound (II-b) from said aqueous medium, and wherein the compound (I-a) is a compound represented by the formula (I-a) (herein referred to as compound (I-a)) :

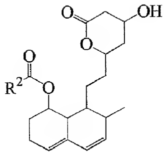


(I-a)

wherein

R^1 represents a hydrogen atom, a substituted or unsubstituted alkyl, or an alkali metal, and R^2 represents a substituted or unsubstituted alkyl, or a substituted or unsubstituted aryl;

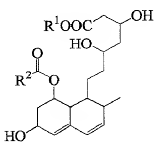
the compound (I-b) is a lactone form of compound (I-a) represented by the formula (I-b) (herein referred to as compound (I-b)):



(I-b)

wherein R^2 has the same definition as the above;

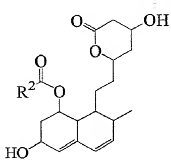
the compound (II-a) is a compound represented by the formula (II-a) (herein referred to as compound (II-a)):



(II-a)

wherein R^1 and R^2 have the same definitions as the above; and

the compound (II-b) is a lactone form of compound (II-a) represented by the formula (II-b) (herein referred to as compound (II-b)):

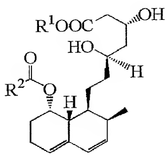


(II-b)

wherein R^2 has the same definition as the above.

2. The process according to claim 1, wherein

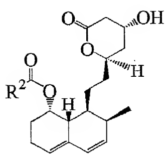
the compound (I-a) is a compound represented by the formula (III-a) (herein referred to as compound (III-a)):



(III-a)

wherein R^1 represents a hydrogen atom, a substituted or unsubstituted alkyl, or an alkali metal, and R^2 represents a substituted or unsubstituted alkyl, or a substituted or unsubstituted aryl;

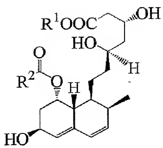
the compound (I-b) is a compound represented by the formula (III-b) (herein referred to as compound (III-b)):



(III-b)

wherein R^2 has the same definition as the above;

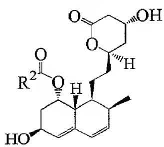
the compound (II-a) is a compound represented by the formula (IV-a) (herein referred to as compound (IV-a)):



(IV-a)

wherein R^1 and R^2 have the same definitions as the above; and

the compound (II-b) is a compound represented by the formula (IV-b) (herein referred to as compound (IV-b)):

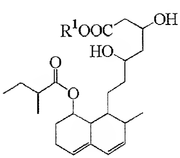


(IV-b)

wherein R^2 has the same definition as the above.

3. The process according to claim 1, wherein

the compound (I-a) is a compound represented by the formula (V-a) (herein referred to as compound (V-a)):

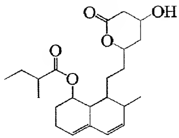


(V-a)

wherein R^1 represents a hydrogen atom, a substituted or unsubstituted alkyl, or an alkali metal;

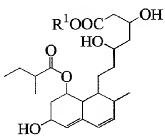
the compound (I-b) is a compound represented by the formula (V-b)(herein referred to

as compound (V-b));



(V-b)

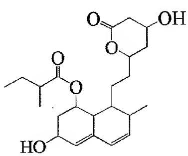
the compound (II-a) is a compound represented by the formula (VI-a) (herein referred to as compound (VI-a)):



(VI-a)

wherein R^1 has the same definition as the above; and

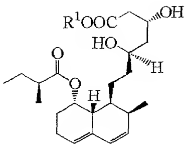
the compound (II-b) is a compound represented by the formula (VI-b) (herein referred to as compound (VI-b)):



(VI-b)

4. The process according to claim 1, wherein

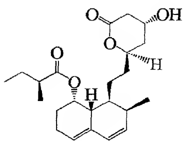
the compound (I-a) is a compound represented by the formula (VII-a) (herein referred to as compound (VII-a)):



(VII-a)

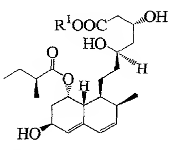
wherein R^1 represents a hydrogen atom, a substituted or unsubstituted alkyl, or an alkali metal;

the compound (I-b) is a compound represented by the formula (VII-b) (herein referred to as compound (VII-b)):



(VII-b)

the compound (II-a) is a compound represented by the formula (VIII-a) (herein referred to as compound (VIII-a)):

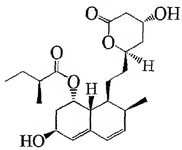


(VIII-a)

wherein R^1 has the same definition as the above; and

the compound (II-b) is a compound represented by the formula (VIII-b) (herein referred

to as compound (VIII-b)):



(VIII-b)

5. The process according to claim 1, wherein the treated product of the culture of the microorganism is a treated product selected from cultured cells; treated products such as dried cells, freeze-dried cells, cells treated with a surfactant, cells treated with an enzyme, cells treated by ultrasonication, cells treated by mechanical milling, cells treated by solvent; a protein fraction of a cell; and an immobilized products of cells or treated cells.

6. The process according to claim 1, wherein the microorganism is selected from those belonging to the genus *Mycobacterium, Corynebacterium, Brevibacterium, Rhodococcus, Gordona, Arthrobacter, Micrococcus, Cellulomonas* and *Sphingomonas.*

7. The process according to claim 1, wherein the microorganism is one selected from *Mycobacterium phlei, Mycobacterium smegmatis, Mycobacterium thermoresistibile, Mycobacterium neoaurum, Mycobacterium parafortuitum, Mycobacterium gilvum, Rhodococcus globerulus, Rhodococcus equi, Rhodococcus erythropolis, Rhodococcus rhodochrous, Rhodococcus rhodnii, Rhodococcus ruber, Rhodococcus coprophilus, Rhodococcus fascians, Gordona amarae, Gordona rubropertinctus, Gordona bronchialis, Gordona sputi, Gordona aichiensis, Gordona terrae, Corynebacterium glutamicum, Corynebacterium mycetoides, Corynebacterium variabilis, Corynebacterium ammoniagenes, Arthrobacter crystallopoietes, Arthrobacter duodecadis, Arthrobacter ramosus, Arthrobacter sulfureus, Arthrobacter aurescens,*

Arthrobacter citreus, *Arthrobacter globiformis*, *Brevibacterium acetylicum*, *Brevibacterium linens*, *Brevibacterium incertum*, *Brevibacterium iodinum*, *Micrococcus luteus*, *Micrococcus roseus*, *Cellulomonas cellulans*, *Cellulomonas cartae*, *Sphingomonas paucimobilis*, *Sphingomonas adhaesiva*, and *Sphingomonas terrae*.

8. The process according to claim 1, wherein the microorganism is one selected from *Mycobacterium phlei* JCM5865, *Mycobacterium smegmatis* JCM5866, *Mycobacterium thermoresistibile* JCM6362, *Mycobacterium neoaurum* JCM6365, *Mycobacterium parafortuitum* JCM6367, *Mycobacterium gilvum* JCM6395, *Rhodococcus globerulus* ATCC25714, *Rhodococcus equi* ATCC21387, *Rhodococcus equi* ATCC7005, *Rhodococcus erythropolis* ATCC4277, *Rhodococcus rhodochrous* ATCC21430, *Rhodococcus rhodochrous* ATCC13808, *Rhodococcus rhodnii* ATCC35071, *Rhodococcus ruber* JCM3205, *Rhodococcus coprophilus* ATCC29080, *Rhodococcus fascians* ATCC12974, *Rhodococcus fascians* ATCC35014, *Gordona amarae* ATCC27808, *Gordona rubropertinctus* IFM-33, *Gordona rubropertinctus* ATCC14352, *Gordona bronchialis* ATCC25592, *Gordona sputi* ATCC29627, *Gordona aichiensis* ATCC33611, *Gordona terrae* ATCC25594, *Corynebacterium glutamicum* ATCC13032, *Corynebacterium glutamicum* ATCC14020, *Corynebacterium glutamicum* ATCC19240, *Corynebacterium mycetoides* ATCC21134, *Corynebacterium variabilis* ATCC15753, *Corynebacterium ammoniagenes* ATCC6872, *Arthrobacter crystallopoietes* ATCC15481, *Arthrobacter duodecadis* ATCC13347, *Arthrobacter ramosus* ATCC13727, *Arthrobacter sulfureus* ATCC19098, *Arthrobacter aurescens* ATCC13344, *Arthrobacter citreus* ATCC11624, *Arthrobacter globiformis* ATCC8010, *Brevibacterium acetylicum* ATCC953, *Brevibacterium linens* ATCC19391, *Brevibacterium linens* ATCC9172, *Brevibacterium incertum* ATCC8363, *Brevibacterium iodinum* IFO3558, *Micrococcus luteus* ATCC4698, *Micrococcus roseus* ATCC186, *Cellulomonas cellulans* ATCC15921, *Cellulomonas cartae* ATCC21681, *Sphingomonas paucimobilis* ATCC29837, *Sphingomonas adhaesiva* JCM7370, and *Sphingomonas terrae* ATCC15098.

9. The process according to claim 1, wherein the microorganism is *Gordona* sp. ATCC19067.

P 21252
FA1522A/US

Docket #: p21252.dc1.doc

Declaration and Power of Attorney for Utility or Design Patent Application
特許出願宣言書

Japanese Language Declaration

私は、下欄に氏名を記載した発明者として、以下のとおり宣言する：

私の住所、郵便の宛先および国籍は、下欄に氏名に続いて記載したとおりであり、

名称の発明に関し、請求の範囲に記載した特許を求める主題の本来の、最初にして唯一の発明者である（一人の氏名のみが下欄に記載されている場合）か、もしくは本来の、最初にして共同の発明者である（複数の氏名が下欄に記載されている場合）と信じ、

上記発明の明細書（下記の欄で x 印がついていない場合は、本書に添付）は、

＿年＿月＿日に提出され、米国出願番号＿＿＿とし、（該当する場合）＿年＿月＿日に訂正されました。又は、

特許協定条約国際出願番号＿＿＿とし、（該当する場合）＿年＿月＿日に訂正されました。

私は、前記のとおり補正した請求の範囲を含む前記明細書の内容を検討し、理解したことを陳述する。

私は、連邦規則法典第 37 編第 1 条 56 項に定義されているとおり、特許資格の有無について重要な情報を開示すべき義務があることを認めます。

私は、合衆国法典第 35 部第 119 条（a-d）項又は第 365 条(b)項に基づく、下記の外国特許出願又は発明者証出願、或いは第 365 条(a)項に基づく、少なくても米国以外の 1 カ国を指名した PCT 国際出願の外国優先権を主張し、更に優先権の主張に係わる基礎出願の出願日前の出願日を有する外国特許出願、又は発明者証出願或るいは PCT 国際出願を以下に“なし”の箱に印をつけることにより明記する：

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name:

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

Process For Producing HMG-CoA Reductase Inhibitors

the specification of which is attached hereto unless the following box is checked:

☒ was filed on 20/Jan/00 as United States Application Number 09/868,924 and was amended on 13/Jul/01 (if applicable) or,

PCT International Application Number PCT/JP00/00245 and was amended on ＿＿＿ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority under Title 35, United States Code §119(a-d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT international application which designated at least one country other than the United States, listed below. I have also identified below, by checking the "No" box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed:

Prior foreign applications
先の外国出願

(Number) (番号)	(Country) (国名)	(Day/Month/Year Filed) (出願の年月日)	Priority claimed 優先権の主張 Yes あり	No なし
11/12392	Japan	20/Jan/99	☒	☐
			☐	☐

☐ その他の外国特許出願番号は別紙の追補優先権欄にて記載する。

☐ Additional foreign application numbers are listed a supplemental priority sheet attached hereto.

Docket #: p21252.dc1.doc

Japanese Language Utility or Design Patent Application Declaration

私は、合衆国法典第 35 部第 119 条(e)項に基づく、下記の合衆国仮特許出願の利益を主張する。

I hereby claim the benefit under Title 35, United States Code §119 (e) of any United States provisional application(s) listed below.

(Application No.) (出願番号)	(Day/Month/Year Filed) (出願の年月日)
(Application No.) (出願番号)	(Day/Month/Year Filed) (出願の年月日)
(Application No.) (出願番号)	(Day/Month/Year Filed) (出願の年月日)

☐ その他の合衆国仮特許出願番号は別紙の追補優先権欄にて記載する。

☐ Additional provisional application numbers are listed on a supplemental priority sheet attached hereto.

私は、合衆国法典第 35 部第 120 条に基づく下記の合衆国特許出願、又は第 365 条(c)項に基づく合衆国を指名した PCT 国際出願の利益を主張し、本願の請求の範囲各項に記載の主題が合衆国法典第 35 部第 112 条第 1 項規定の態様で、先の合衆国特許出願又は PCT 国際出願に開示されていない限度において、先の出願の出願日と本願の国内出願日又は PCT 国際出願日の間に有効となった連邦規則法典第 37 部第 1 章第 56 条に記載の特許要件に所要の情報を開示すべき義務を有することを認める。

I hereby claim the benefit under Title 35, United States Code §120 of any United States application(s), or §365(c) of any PCT international application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of Title 35, United States Code §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

(Application No.) (出願番号)	(Day/Month/Year Filed) (出願の年月日)	(現況) (特許済み、係属中 放棄済み)	(Status) (patented, pending, abandoned)
(Application No.) (出願番号)	(Day/Month/Year Filed) (出願の年月日)	(現況) (特許済み、係属中 放棄済み)	(Status) (patented, pending, abandoned)

☐ その他の合衆国又は国際特許出願番号は別紙の追補優先権欄にて記載する。

☐ Additional U.S. or international application numbers are listed on a supplemental priority sheet attached hereto.

私は、ここに自己のに知識に基づいて行った陳述が全て真実であり、自己の有する情報および信ずるところに従って行った陳述が真実であると信じ、さらに故意に虚偽の陳述等を行った場合、合衆国法典第 18 部第 1001 条により、罰金もしくは禁　に処せられるか、またはこれらの刑が併科され、またかかる故意による虚偽による陳述が本願ないし本願に対して付与される特許の有効性を損なうことがあることを認識して、以上の陳述を行ったことを宣言する。

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

私、下記署名者は、ここに記載の米国弁護士または代理人に本出願に関し特許商標庁にて取られるいかなる行為に関して、同米国弁護士又は代理人が私に直接連絡なしに私の外国弁護士或るいは法人代表者からの指示を受け取り、それに従うようここに委任する。この指示を出す者が変更の場合には、ここに記載の米国弁護士又は代理人にその旨通知される。

The undersigned hereby authorizes the U.S. attorney or agent named herein to accept and follow instructions from either his foreign patent agent or corporate representative, if any, as to any action to be taken in the Patent and Trademark Office regarding this application without direct communication between the U.S. attorney or agent and the undersigned. In the event of a change in the persons from whom instructions may be taken, the U.S. attorney or agent named herein will be so notified by the undersigned.

貴社 Ref. 11179W01
当所 Ref: FA1522A/US

Docket #: p21252.dc1.doc

Japanese Language Utility or Design Patent Application Declaration

委任状：　私は、下記発明者として、下記に明記された顧客番号を伴う以下の弁護士又は、代理人をここに選任し、本願の手続きを遂行すること並びにこれに関する一切の行為を特許商標庁に対して行うことを委任する。そして全ての通信はこの顧客番号宛に発送される。

顧客番号　7055

現在委任された弁護士は下記の通りである。

POWER OF ATTORNEY: As a named inventor, I hereby appoint the attorney(s) and/or agent(s) associated with the Customer Number provided below to prosecute this application and transact all business in the Patent and Trademark Office connected therewith, and direct that all correspondence be addressed to that Customer Number:

CUSTOMER NUMBER 7055

The appointed attorneys presently include:

Neil F. Greenblum	Reg. No. 28,394	Stephen M. Roylance	Reg. No. 31,296
Bruce H. Bernstein	Reg. No. 29,027	William E. Lyddane	Reg. No. 41,568
James L. Rowland	Reg. No. 32,674	William Pieprz	Reg. No. 33,630
Arnold Turk	Reg. No. 33,094	Leslie J. Paperner	Reg. No. 33,329

Address: **GREENBLUM & BERNSTEIN, P.L.C.**
1941 Roland Clarke Place
Reston, VA 20191

直接電話連絡先：　Direct Telephone Calls to:

GREENBLUM & BERNSTEIN, P.L.C.
(703) 716-1191

唯一のまたは第一の発明者の氏名　1-00	Full name of sole or first inventor Shin-ichi HASHIMOTO
同発明者の署名　日付	Inventor's signature　Date Shin-ichi Hashimoto　September 13, 2001
住所	Residence Hofu-shi, Yamaguchi, Japan JPX
国籍	Citizenship Japan
郵便の宛先	Post Office Address c/o Tokyo Research Laboratories, Kyowa Hakko Kogyo Co., Ltd.
	6-6, Asahi-machi 3-chome, Machida-shi, Tokyo 194-8533, Japan
第二の共同発明者の氏名（該当する場合）　2-00	Full name of second joint inventor, if any Yoshiyuki YONETANI
同第二共同発明者の署名　日付	Second Inventor's signature　Date Yoshiyuki Yonetani　September 13, 2001
住所	Residence Machida-shi, Tokyo, Japan JPX
国籍	Citizenship Japan
郵便の宛先	Post Office Address c/o Tokyo Research Laboratories, Kyowa Hakko Kogyo Co., Ltd.
	6-6, Asahi-machi 3-chome, Machida-shi, Tokyo 194-8533, Japan

（第三またはそれ以降の共同発明者に対しても同様な情報および署名を提供すること。）

(Supply similar information and signature for third and subsequent joint inventors.)

Japanese Language Utility or Design Patent Application Declaration

第三の共同発明者の氏名（該当する場合） 300	Full name of third joint inventor, if any Akio OZAKI
共同発明者の署名 日付	Third Inventor's signature Akio Ozaki Date September 13, 2001
住所	Residence Hofu-shi, Yamaguchi, Japan JPX
国籍	Citizenship Japan
郵便の宛先	Post Office Address c/o Technical Research Laboratories, Kyowa Hakko Kogyo Co., Ltd.
	1-1, Kyowa-cho, Hofu-shi, Yamaguchi 747-8522, Japan
第四の共同発明者の氏名（該当する場合）	Full name of fourth joint inventor, if any
共同発明者の署名 日付	Fourth Inventor's signature Date
住所	Residence
国籍	Citizenship
郵便の宛先	Post Office Address
第五の共同発明者の氏名（該当する場合）	Full name of fifth joint inventor, if any
共同発明者の署名 日付	Fifth Inventor's signature Date
住所	Residence
国籍	Citizenship
郵便の宛先	Post Office Address
第六の共同発明者の氏名（該当する場合）	Full name of sixth joint inventor, if any
共同発明者の署名 日付	Sixth Inventor's signature Date
住所	Residence
国籍	Citizenship
郵便の宛先	Post Office Address

（それ以降の共同発明者に対しても同様な情報および署名を提供すること。）

(Supply similar information and signature for subsequent joint inventors.)